

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
4 April 2002 (04.04.2002)

PCT

(10) International Publication Number
WO 02/27032 A1

- (51) International Patent Classification⁷: **C12Q 1/68**
- (21) International Application Number: **PCT/US01/30455**
- (22) International Filing Date:
28 September 2001 (28.09.2001)
- (25) Filing Language: **English**
- (26) Publication Language: **English**
- (30) Priority Data:
60/236,179 29 September 2000 (29.09.2000) US
09/721,918 27 November 2000 (27.11.2000) US
- (71) Applicant: **MYRIAD GENETICS, INC.** [US/US]; 320 Wakara Way, Salt Lake City, UT 84108 (US).
- (72) Inventors: **TUSNEEM, Nadeem**; 4751 W. Summit Valley Drive, West Jordan, UT 84088 (US). **PRUSS, Dmitry**; 7311 Hickory Hill, Salt Lake City, UT 84121 (US). **SHEN, Min-Jui, Richard**; 1043 English Oaks Drive, Arcadia, CA 91006 (US). **BHATNAGAR, Satish, K.**; 9141 South Wedgefield Drive, Sandy, UT 84093 (US).
- (74) Agents: **IHNEN, Jeffrey, L.** et al.; Rothwell, Figg, Ernst & Manbeck, P.C., Suite 701-B, 555 13th Street, N.W., Washington, DC 20004 (US).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:
— with international search report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/27032 A1

(54) Title: **METHOD FOR EQUALIZING BAND INTENSITIES ON SEQUENCING GELS**

(57) Abstract: A method for normalizing the intensity of G bands in sequencing methods which utilize dITP is presented. The use of dITP normally results in decreased intensities of G bands which occur after A bands, i.e., in the sequence AG. It has been discovered that the use of ddITP in place of ddGTP or in conjunction with ddGTP helps to normalize the intensity of the G bands following A bands. This aids in preventing errors in reading sequencing chromatograms and allows for extended reads of sequencing chromatograms as compared to methods which utilize dITP without the use of ddITP.

TITLE OF THE INVENTION**METHOD FOR EQUALIZING BAND INTENSITIES ON SEQUENCING GELS****BACKGROUND OF THE INVENTION**

The present invention is directed to a method for normalizing the intensity of G bands in sequencing methods which utilize dITP is presented. More specifically, the present invention is directed to the use of ddITP in place of ddGTP or in conjunction with ddGTP to normalize the intensity of the G bands following A bands. This normalization aids in preventing errors in reading sequencing chromatograms and allows for extended reads of sequencing chromatograms as compared to methods which utilize dITP without the use of ddITP.

The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated by reference, and for convenience are referenced in the following text by author and date and are listed alphabetically by author in the bibliography.

Resolution of DNA sequences by the method of Sanger et al. (1977) becomes difficult in regions of dyad symmetry with high G+C content. These regions form secondary structures within the dideoxy-terminated product resulting in gel compressions during electrophoresis. Replacing deoxyguanosine triphosphate (dGTP) with deoxyinosine triphosphate (dITP) alleviates this problem (Tabor and Richardson, 1987): dGTP-dCTP forms three hydrogen bonds whereas dITP-dCTP forms two hydrogen bonds (Mills and Kramer, 1979). A number of other compounds have also been used to remove compressions. These include 7-deaza-2'-deoxyguanosine-5'-triphosphate (Mizusawa et al., 1986) (which like dITP is also used in place of dGTP) and N-4-methyl-2'-deoxycytidine-5'-triphosphate, which is used in place of deoxycytidine triphosphate (dCTP) (Li et al., 1993). Using 7-deaza-ATP with 7-deaza-GTP has also been shown to help reduce compressions (Jensen et al., 1991). However, these components in sequencing reactions have not been as effective as dITP in reducing compressions or have resulted in appearance of banding artifacts caused by premature termination (Li et al., 1993; Ausubel et al., 1999).

Non-biochemical methods have also been suggested in alleviating compressions and have resulted in a lesser degree of success than have biochemical means. The most common non-biochemical method is to add formamide to sequencing gels in order to promote denaturation of secondary structures as dideoxy-terminated molecules migrate through the gel (Rocheleau et al., 1992). A second method for removing compressions requires running a sequencing gel at higher temperatures than the temperature at which they are commonly run (Ausubel et al., 1999).

However, this approach is not widely used as glass gel plates crack at higher temperatures and resolution suffers. Another method, not widely used, is to modify the C residues chemically so they can no longer form GC base pairs. This can be accomplished by treating the sequencing products with bisulfite or a mixture of bisulfite and methoxyamine (Ambartsumyan and Mazo, 1980; Hayatsu, 1976).

The best candidate for removal of compressions is dITP which is widely used. A number of problems have been associated with use of dITP and solutions have been suggested in the literature. First, the rate of dITP incorporation is slower than is the rate of dGTP incorporation causing the reaction to terminate quickly thereby resulting in inability to resolve bases far from the primer (McCrea et al., 1993). To overcome this problem, ddGTP concentration can be reduced in the mixture. A second problem associated with dITP is that while the sequencing enzyme uses dITP efficiently, it has a tendency to stall in sequencing reactions and produce sequencing ladders that have a higher frequency of bands in all four lanes (as in the case of use of radioactive labels). Adding terminal deoxynucleotidyl transferase to the reaction can eliminate this problem (Fawcett and Bartlett, 1990).

Another significant and yet unresolved problem associated with the use of dITP is the frequent decrease in amplitude of G peaks following an A peak (Figure 1). Also, the amplitude of G in a string of two or more Gs is low. This is most noticeable when the first G is preceded by an A. This results in problems in resolution of G peaks far from the primer and in reactions where the overall signal intensity is low and could result in erroneous base calling. This phenomenon has been observed both in dye terminator and dye primer sequencing chemistries. An explanation for this result could be that dITP gets incorporated at a higher frequency than does ddGTP after dATP has been incorporated.

Thus, it is an object of the present invention to provide a method for DNA sequencing which does not have these problems and which allows for longer and more accurate reads of sequencing gels.

SUMMARY OF THE INVENTION

The present invention is directed to DNA sequencing reactions which utilize dITP to minimize band compressions on gels. In accordance with the present invention, ddITP or a combination of ddITP and ddGTP is used as chain terminators in sequencing reactions to increase the amplitude of G peaks following A peaks. This use of ddITP or combination of ddITP with

ddGTP results in the G peaks which follow A peaks being of greater amplitude than they are in the absence of using ddITP and more similar to the amplitude of neighboring peaks. This use of ddITP or combination of ddITP with ddGTP allows longer and more accurate reads of each sequencing gel.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a sequencing chromatogram from a dye terminator sequencing reaction showing the results of a reaction utilizing dITP with ddGTP and illustrating the decreased intensity of G peaks following A peaks.

Figure 2A is a sequencing chromatogram showing the results of a reaction utilizing dGTP and ddGTP (Experiment 1A) and illustrating the presence of band compressions.

Figure 2B is a sequencing chromatogram showing the results of a reaction utilizing dITP and ddGTP (Experiment 1B) and illustrating that the band compressions of Figure 2A are eliminated but at the cost of decreased intensity of G peaks following A peaks.

Figure 3A is a sequencing chromatogram showing the results of a reaction utilizing dGTP and ddITP (Experiment 2A) illustrating that ddITP is not useful for eliminating band compressions.

Figure 3B is a sequencing chromatogram showing the results of a reaction utilizing dITP and ddITP (Experiment 2B) illustrating that this combination minimizes band compressions and also helps to normalize the intensity of G peaks following A peaks.

Figure 4A is a sequencing chromatogram showing the results of a reaction utilizing dGTP and ddGTP (Experiment 1A) illustrating the presence of compressions but with relatively even peak intensities in the regions without compressions.

Figure 4B is a sequencing chromatogram showing the results of a reaction utilizing dITP and ddGTP (Experiment 1B) illustrating the minimization of band compressions but at the cost of decreased G peak intensities following A bands.

Figure 4C is a sequencing chromatogram showing the results of a reaction utilizing dITP and ddITP (Experiment 2B) illustrating the minimization of band compressions while overcoming the decrease of G peak intensities following an A peak.

Figure 4D is a sequencing chromatogram showing the results of a reaction utilizing dITP and ddITP plus ddGTP (Experiment 3) illustrating the minimization of band compressions while obtaining even G peaks following A peaks as compared to Figure 4B.

Figures 5A-D are sequencing chromatograms of the same template as in Figures 4A-D except that a different region is shown.

DETAILED DESCRIPTION OF THE INVENTION

The interpretation of DNA sequences by the method of Sanger et al. (1977) can be difficult at areas of dyad symmetry rich in G+C content. These regions can produce secondary structures within the dideoxy-terminated product resulting in gel compressions during electrophoresis. A variety of methods have been used to eliminate compressions including substitution of dITP or 7-deaza-2'-deoxyguanosine-5'-triphosphate for dGTP and N-4 methyl-2'-deoxycytidine-5'-triphosphate for dCTP to reduce the number of hydrogen bonds between G and C residues. 7-deaza-dATP in combination with 7-deaza-dGTP is also useful in resolving compressions. However some of these reactions tend to have banding artifacts caused by premature termination of the polymerase. Other suggestions involve running sequencing gels at the highest possible temperature or inclusion of formamide in the sequencing gel to promote denaturation of secondary structures. Another method not widely used is to chemically modify C residues so that they can no longer engage in the formation of G-C base pairs.

The most widely used method for minimizing gel band compressions on sequencing gels is to use dITP in place of dGTP. Although this method eliminates many of the band compressions, it results in a new problem, this being that the amplitude (as measured on sequencing gels using fluorescent labels) of G bands is relatively small as compared to neighboring bands when the G immediately follows an A. This decreased amplitude increases the possibility that the G band will be overlooked, especially when the sequence is far from the primer and the intensities (amplitudes) of all bands are decreasing. The present invention is directed to the use of ddITP in the G termination reaction to overcome this problem.

Use of ddITP in research has been limited to study of viral polymerase activities (Boyer and Hughes, 1995; Strahl and Blackburn, 1994). The only comment regarding ddITP and sequencing in the literature is that ddGTP, and not ddITP, should be used in sequencing reactions (Bankier and Barrell, 1983). In the present disclosure, it is shown that ddITP can be used in sequencing reactions along with dITP to overcome the decreased G band intensity which occurs with the sequence AG. Furthermore, a mixture of ddITP and ddGTP in the G termination sequencing reaction can also be used.

Several different kits are commercially available for performing sequencing. The problems discussed above concerning band compressions on sequencing gels and decreased peak intensity will appear to varying extents with these kits. These kits can be modified to use ddITP or a combination of ddITP and ddGTP to aid in minimizing these problems.

EXAMPLES

The present invention is further detailed in the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described below are utilized.

The following Examples illustrate the method of incorporating ddITP into sequencing reactions to help equalize the intensities of G bands which follow A bands with the intensities of the bands neighboring the G bands. It should be noted that the products obtained from the sequencing reactions using the invention can be analyzed by any appropriate means, e.g., the products can be electrophoresed on a polyacrylamide gel or they can be analyzed by use of capillary tubes.

EXAMPLE 1

Illustration of the Prior Art Problems

Sequencing reactions using dITP in place of dGTP in order to remove compressions have a very characteristic phenomenon. This phenomenon was observed in dye terminator sequencing using the PE dye terminator kit (Figure 1). The amplitudes of G following an A are reduced. Also notice that the first G peak following an A peak has a lower amplitude than does the second G peak. This problem was also noticed in sequencing reactions using dye primer chemistry during experiments aimed at reducing compressions. Two sets of experiments were set up simultaneously. Experiment 1A involved four reactions using dGTP, dATP, dCTP, and dTTP as deoxyribonucleotides for extension (Table 1A). Experiment 1B used dITP, dATP, dCTP, and dTTP as the deoxyribonucleotides for extension (Table 1B). The terminating dideoxynucleotide for the G reaction was ddGTP and was used in both sets of experiments.

TABLE 1A

Reaction Conditions for dGTP and ddGTP Sequencing Reactions

Experiment Set 1A	"G" termination reaction	"A" termination reaction	"T" termination reaction	"C" termination reaction
dGTP, dATP, dTTP, dCTP	45 μ M	45 μ M	45 μ M	45 μ M
ddGTP	0.37 μ M	---	---	---
ddATP	---	0.37 μ M	---	---
ddTTP	---	---	0.37 μ M	---
ddCTP	---	---	---	0.37 μ M
Taq FS Polymerase	0.225 units/ μ L	0.225 units/ μ L	0.225 units/ μ L	0.225 units/ μ L
"G" Big Dye primer	40 μ M	---	---	---
"A" Big Dye primer	---	40 μ M	---	---
"T" Big Dye primer	---	---	40 μ M	---
"C" Big Dye primer	---	---	---	40 μ M
Sequencing Buffer	1X	1X	1X	1X
Template	10 ng	10 ng	10 ng	10 ng

TABLE 1B

Reaction Conditions for dTTP and ddGTP Sequencing Reactions

Experiment Set 1B	"G" termination reaction	"A" termination reaction	"T" termination reaction	"C" termination reaction
dTTP, dATP, dTTP, dCTP	45 μ M	45 μ M	45 μ M	45 μ M
ddGTP	0.07 μ M	---	---	---
ddATP	---	0.37 μ M	---	---
ddTTP	---	---	0.37 μ M	---
ddCTP	---	---	---	0.37 μ M
Taq FS Polymerase	0.225 units/ μ L	0.225 units/ μ L	0.225 units/ μ L	0.225 units/ μ L
"G" Big Dye primer	40 μ M	---	---	---
"A" Big Dye primer	---	40 μ M	---	---
"T" Big Dye primer	---	---	40 μ M	---
"C" Big Dye primer	---	---	---	40 μ M
Sequencing Buffer	1X	1X	1X	1X
Template	10 ng	10 ng	10 ng	10 ng

The composition of the 1X sequencing buffer used in all of the experiments in this disclosure is 50 mM Tris-HCl pH 9.0, 3 mM MgCl₂, and 0.01% Tween 20. The "Big Dye Primers" are from Perkin Elmer Biosystems (Foster City, California). The reactions were cycle-sequenced for 32 cycles (94°C for 30 seconds, 50°C for 30 seconds, and 60°C for 3 minutes). The four termination reactions from Experiment 1A were consolidated and precipitated with 70% ethanol. The same was repeated for the four sequencing reactions containing dITP as shown in Table 1B. The pellets were resuspended in 10 µL water prior to loading on to capillaries. Samples were run on a MegaBace capillary sequencer and data was analyzed using Myriad Genetics sequencing software. Results of the experiments are shown in Figures 2A, 2B, 4A, 4B, 5A and 5B. Sequencing reactions using dGTP show compressions and show no variation in amplitudes of "G" peaks (Figures 2A, 4A and 5A). Sequencing reactions using dITP resolve the compressions but show variation in "G" peak heights that are incorporated after dATP, suggesting a preference of incorporation of dITP over ddGTP after an incorporation of dATP (Figures 2B, 4B and 5B).

EXAMPLE 2

DNA Sequencing Reactions Using ddITP

In this experiment ddGTP was replaced with ddITP in dye primer sequencing reactions. ddITP was used with dNTP mixtures containing dGTP or dITP. For each set of experiments, four termination reactions were set up (labeled "G", "A", "T", and "C"). ddITP was used in Experiment 2A with dGTP as the extension deoxyribonucleotide and in Experiment 2B with dITP as the extension deoxyribonucleotide. Setup of reactions is described in Tables 2A and 2B.

TABLE 2A

Reaction Conditions for Sequencing Reactions Involving dGTP and ddITP

Experiment Set 2A	"G" termination reaction	"A" termination reaction	"T" termination reaction	"C" termination reaction
dGTP, dATP, dTTP, dCTP	45 µM	45 µM	45 µM	45 µM
ddITP	0.37 µM	---	---	---
ddATP	---	0.37 µM	---	---
ddTTP	---	---	0.37 µM	---
ddCTP	---	---	---	0.37 µM
Taq FS Polymerase	0.225 units/µL	0.225 units/µL	0.225 units/µL	0.225 units/µL

8

"G" Big Dye primer	40 μ M	---	---	---
"A" Big Dye primer	---	40 μ M	---	---
"T" Big Dye primer	---	---	40 μ M	---
"C" Big Dye primer	---	---	---	40 μ M
Sequencing Buffer	1X	1X	1X	1X
Template	10 ng	10 ng	10 ng	10 ng

TABLE 2B

Reaction Conditions for Sequencing Reactions Involving dITP and ddITP

Experiment Set 2B	"G" termination reaction	"A" termination reaction	"T" termination reaction	"C" termination reaction
dITP, dATP, dTTP, dCTP	45 μ M	45 μ M	45 μ M	45 μ M
ddITP	0.37 μ M	---	---	---
ddATP	---	0.37 μ M	---	---
ddTTP	---	---	0.37 μ M	---
ddCTP	---	---	---	0.37 μ M
Taq FS Polymerase	0.225 units/ μ L	0.225 units/ μ L	0.225 units/ μ L	0.225 units/ μ L
"G" Big Dye primer	40 μ M	---	---	---
"A" Big Dye primer	---	40 μ M	---	---
"T" Big Dye primer	---	---	40 μ M	---
"C" Big Dye primer	---	---	---	40 μ M
Sequencing Buffer	1X	1X	1X	1X
Template	10 ng	10 ng	10 ng	10 ng

The reactions were cycle sequenced for 32 cycles (94°C for 30 seconds, 50°C for 30 seconds, and 60°C for 3 minutes). The four termination reactions from each of Experiments 2A and 2B were pooled and precipitated as described for Experiment 1. Samples were run on a MegaBace capillary sequencer and data were analyzed using Myriad Genetics sequencing software. The combination of dGTP and ddITP did not resolve compressions (Figure 3A). The combination of dITP and ddITP resulted in resolution of compressions (Figure 3B) and showed sequencing quality similar to that obtained using dITP and ddGTP but with more even G intensities (compare Figure 4B to Figure 4C and Figure 5B to Figure 5C). The insertion of ddGTP after dATP was not affected as was seen in the case of sequencing with dITP and ddGTP (Experiment 1B). However, there is a slight decrease in G intensity following a T.

EXAMPLE 3

Use of ddITP and ddGTP mixture in sequencing reactions

In order to increase the amplitude of "G" peaks after "A" and/or "T" peaks, a combination of ddGTP and ddITP was used to terminate G reactions in sequencing. For Experiment 3, four sets of G, A, T and C reactions were prepared as described in Table 3 below. The reactions were then cycle-sequenced for 32 cycles (94°C for 30 seconds, 50°C for 30 seconds, and 60°C for 3 minutes). The four termination reactions from Experiment 3 were consolidated and precipitated with 70% ethanol as described above. The pellets were resuspended in 10 µL water. Samples were run on a Megabace capillary sequencer and data were analyzed using Myriad Genetics sequencing software.

TABLE 3

Experimental Conditions for dITP and ddITP/ddGTP Sequencing Reactions

Experiment Set 3	"G" termination reaction	"A" termination reaction	"T" termination reaction	"C" termination reaction
dITP, dATP, dTTP, dCTP	45 µM	45 µM	45 µM	45 µM
ddITP	0.18 µM	---	---	---
ddATP	---	0.37 µM	---	---
ddTTP	---	---	0.37 µM	---
ddCTP	---	---	---	0.37 µM
ddGTP	0.035 µM	---	---	---
Taq FS Polymerase	0.225 units/µL	0.225 units/µL	0.225 units/µL	0.225 units/µL
"G" Big Dye primer	40 µM	---	---	---
"A" Big Dye primer	---	40 µM	---	---
"T" Big Dye primer	---	---	40 µM	---
"C" Big Dye primer	---	---	---	40 µM
Sequencing Buffer	1X	1X	1X	1X
Template	10 ng	10 ng	10 ng	10 ng

The results of this experiment are presented in Figures 4 and 5 (these show different regions of the same sequencing runs). In Experiment 3 (dITP + ddITP + ddGTP), the reduction of amplitude of the G peak after A or T incorporation was less (Figures 4D and 5D) as compared to Experiment 2B (dITP + ddITP; Figures 4C and 5C) and Experiment 1B (dITP + ddGTP; Figures 4B and 5B), ie., the intensity of the G bands was more even to the neighboring bands.. This result

allows one to read longer sequences than can be read in the absence of ddITP because the G intensity is increased and the G bands are not lost in the background as readily as the signal begins to fade, especially at long distances from the primer sequence.

While the invention has been disclosed in this patent application by reference to the details of preferred embodiments of the invention, it is to be understood that the disclosure is intended in an illustrative rather than in a limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, within the spirit of the invention and the scope of the appended claims.

BIBLIOGRAPHY

-) Ambartsumyan NS and Mazo AM (1980). *FEBS Lett.* 114:265-268.
- Ausubel FM, et al. (eds.) (1999). *Current Protocols in Molecular Biology*. Chapter 7: DNA Sequencing. Unit 7.4A: DNA Sequencing by the dideoxymethod. (John Wiley and Sons, Inc., New York).
- Bankier AT and Barrell BG (1983). *Techniques in Life Sciences, B5: Nucleic Acid Biochemistry* (RA Flavell, ed.), pp. 1-34 (Elsevier/North Holland, Limerick, Ireland).
- Boyer PL and Hughes SH (1995). *Antimicrob. Agents Chemother.* 39:1624-1628.
- Fawcett TW and Bartlett SG (1990). *Biotechniques* 7:700-708.
- Hayatsu H (1976). *Prog. Nucl. Acid Res. Mol. Biol.* 16:75-124.
- Jensen MA, et al. (1991). *DNA Seq.* 1:233-239.
-) Li S, et al. (1993). *Nucl. Acids Res.* 21:2709-2714.
- McCrea KW, et al. (1993). *Biotechniques* 15:843-844.
- Mills DR and Kramer FR (1979). *Proc. Natl. Acad. Sci. USA* 76:2232-2235.
- Mizusawa S, et al. (1986). *Nucl. Acids Res.* 14:1319-1324.
- Rocheleau MJ, et al. (1992). *Electrophoresis* 13:484-486.
-) Sanger F, et al. (1977). *Proc. Natl. Acad. Sci. USA* 74:5463-5467.
- Strahl C and Blackburn EH (1994). *Nucl. Acids Res.* 22:893-900.
- Tabor S and Richardson C (1987). *Proc. Natl. Acad. Sci. USA* 84:4767-4771.

WHAT IS CLAIMED IS:

1. A method for increasing the intensity of a G band in a sequencing analysis when said G band immediately follows an A band, comprising utilizing ddITP in a sequencing reaction which includes dITP and analyzing said sequencing reaction.
2. The method of claim 1, wherein ddITP is utilized in the absence of ddGTP.
3. The method of claim 1, wherein ddITP is utilized together with ddGTP.
4. The method of claim 1, wherein said sequencing reaction utilizes Taq FS polymerase.
5. The method of claim 2, wherein said sequencing reaction utilizes Taq FS polymerase.
6. The method of claim 3, wherein said sequencing reaction utilizes Taq FS polymerase.
7. The method of any one of claims 1-6, wherein said analyzing is by gel electrophoresis.
8. The method of any one of claims 1-6, wherein said analyzing is by capillary electrophoresis.

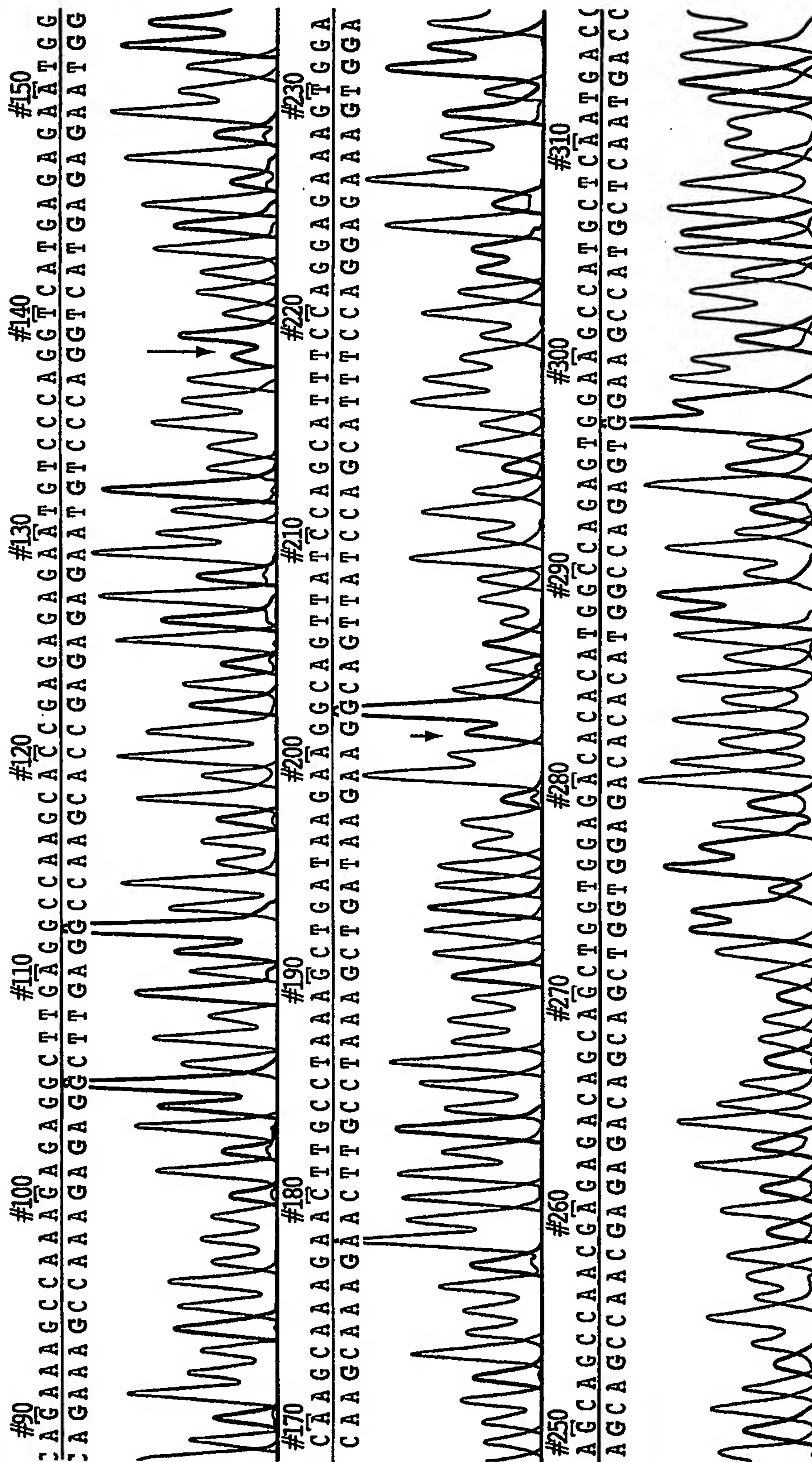


FIG. 1

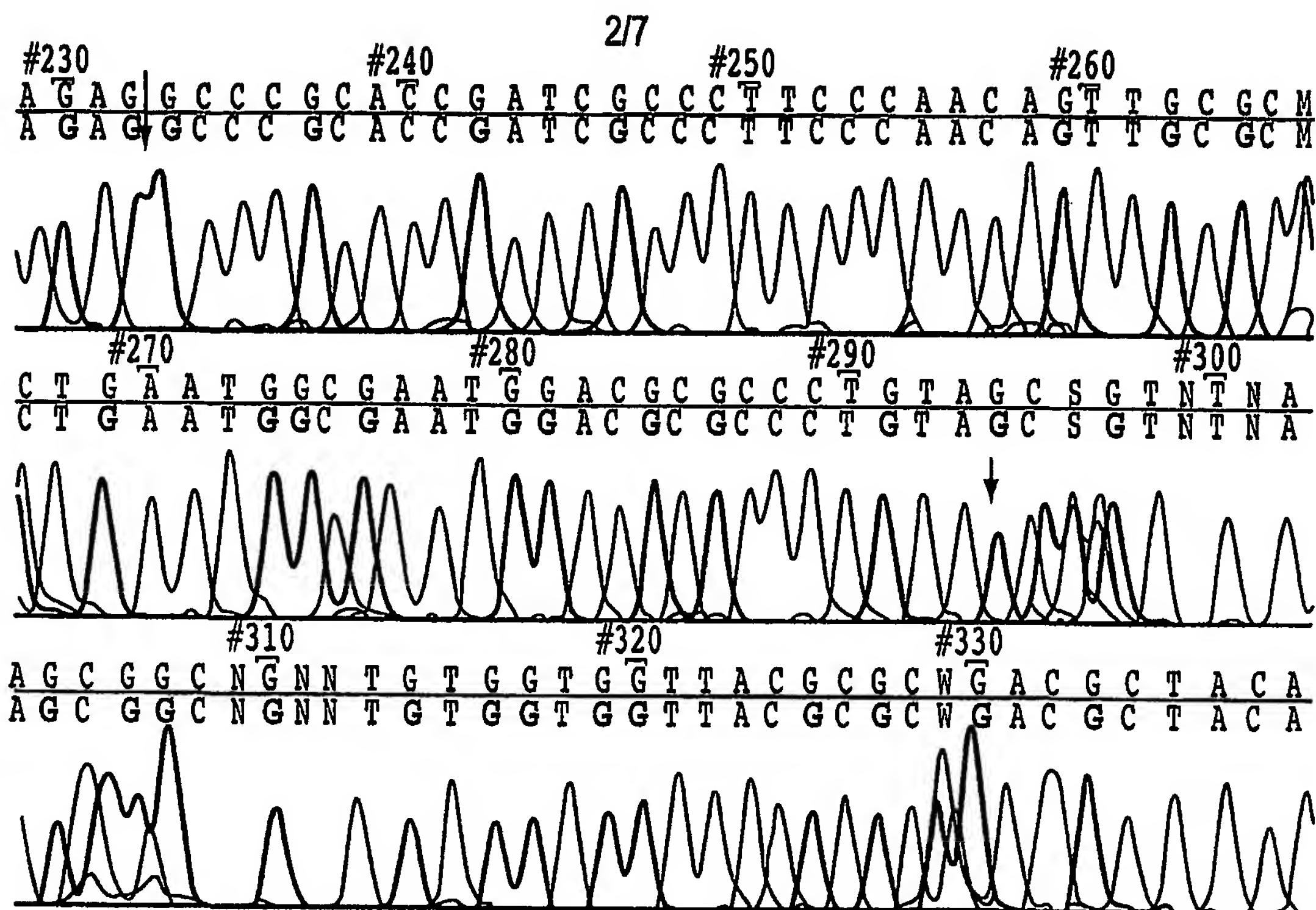


FIG. 2A

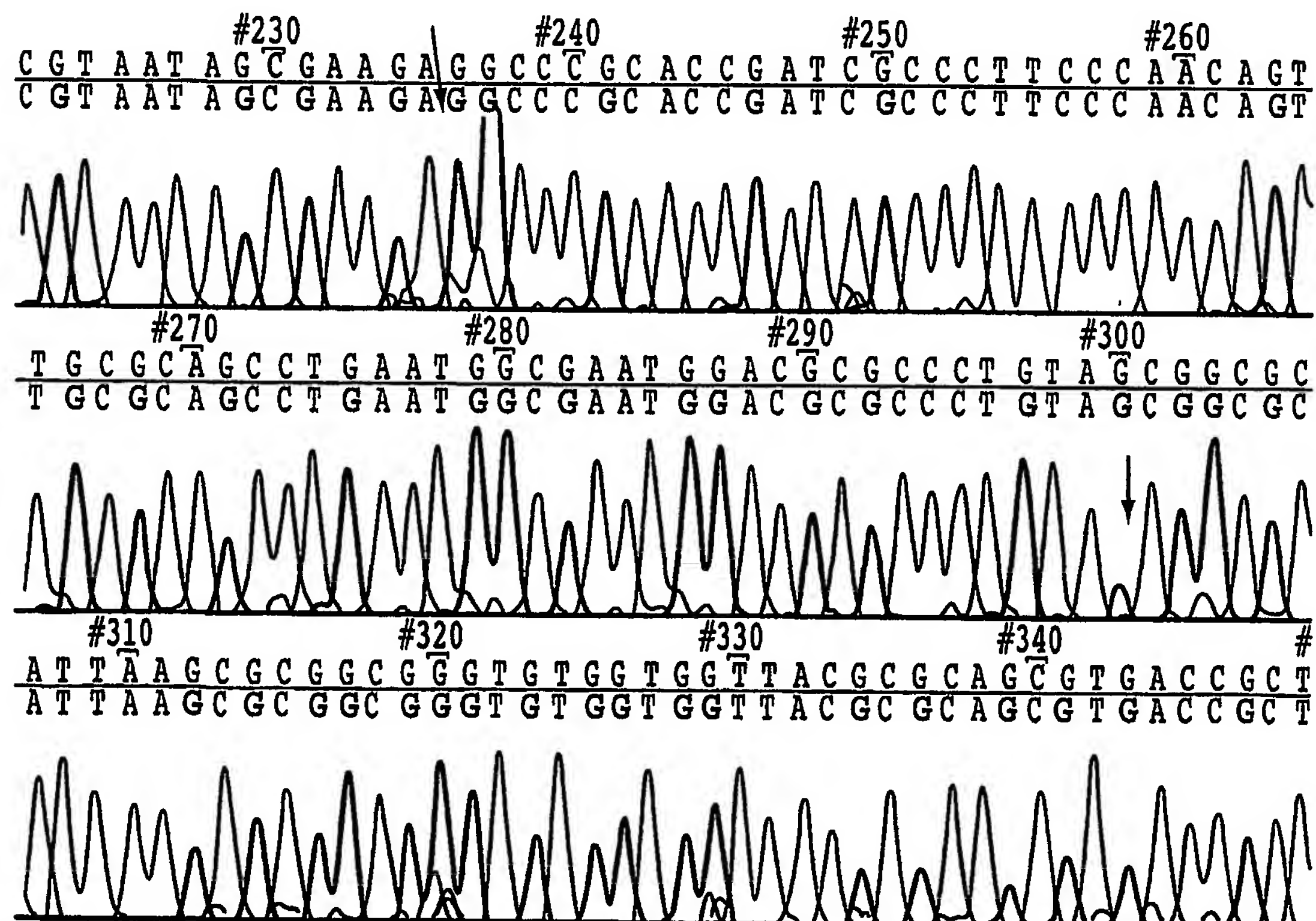


FIG. 2B

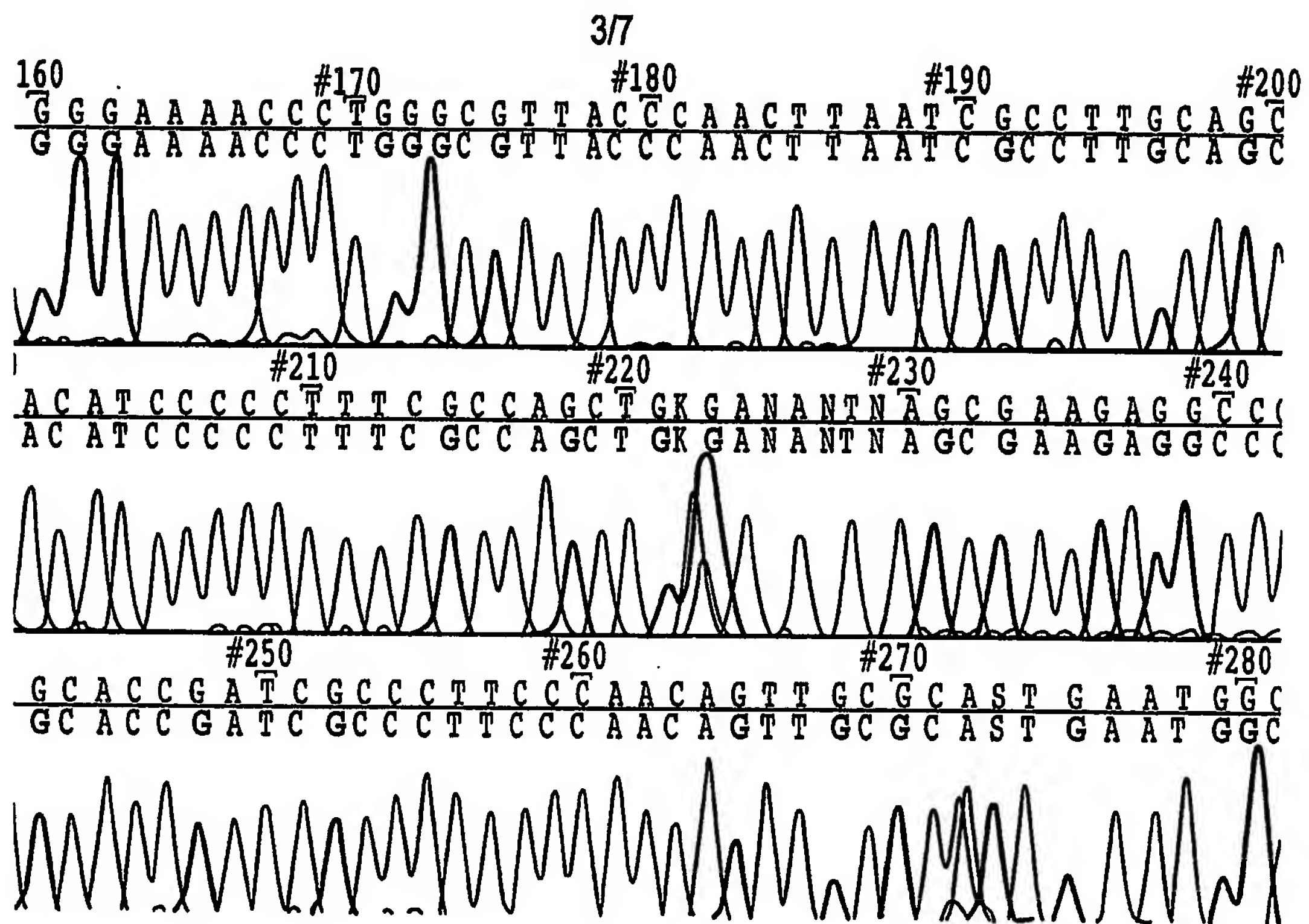


FIG. 3A

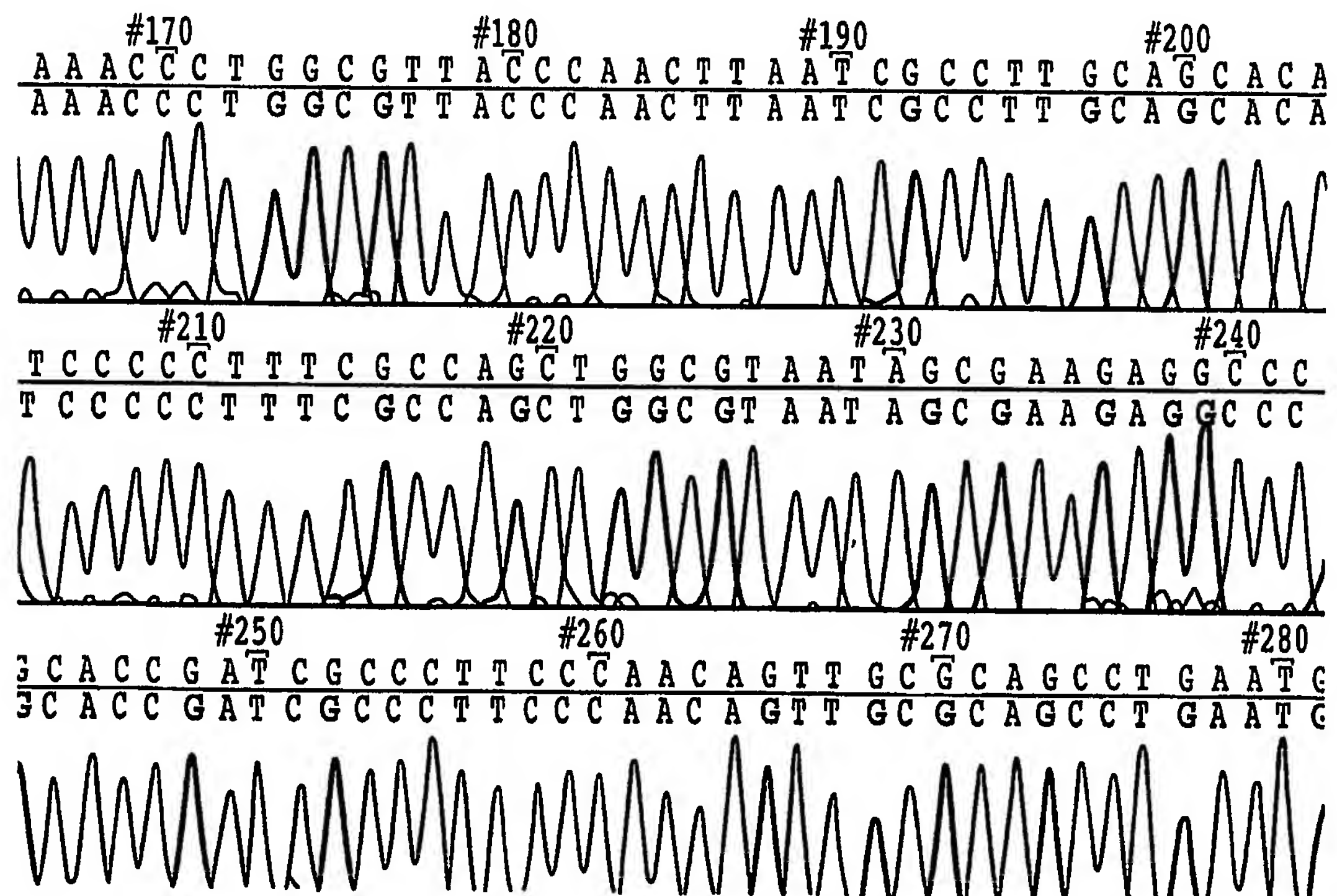


FIG. 3B

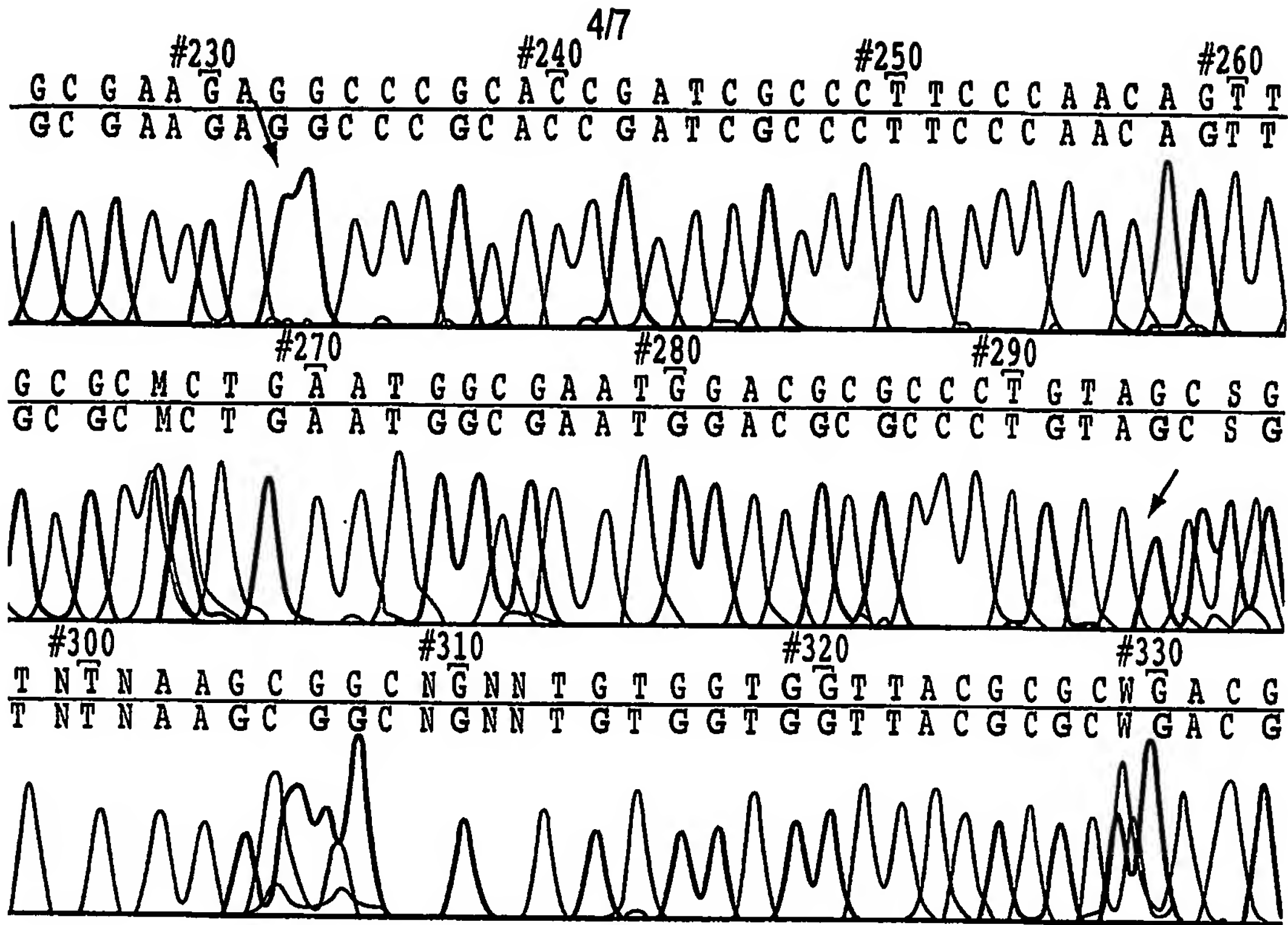


FIG. 4A

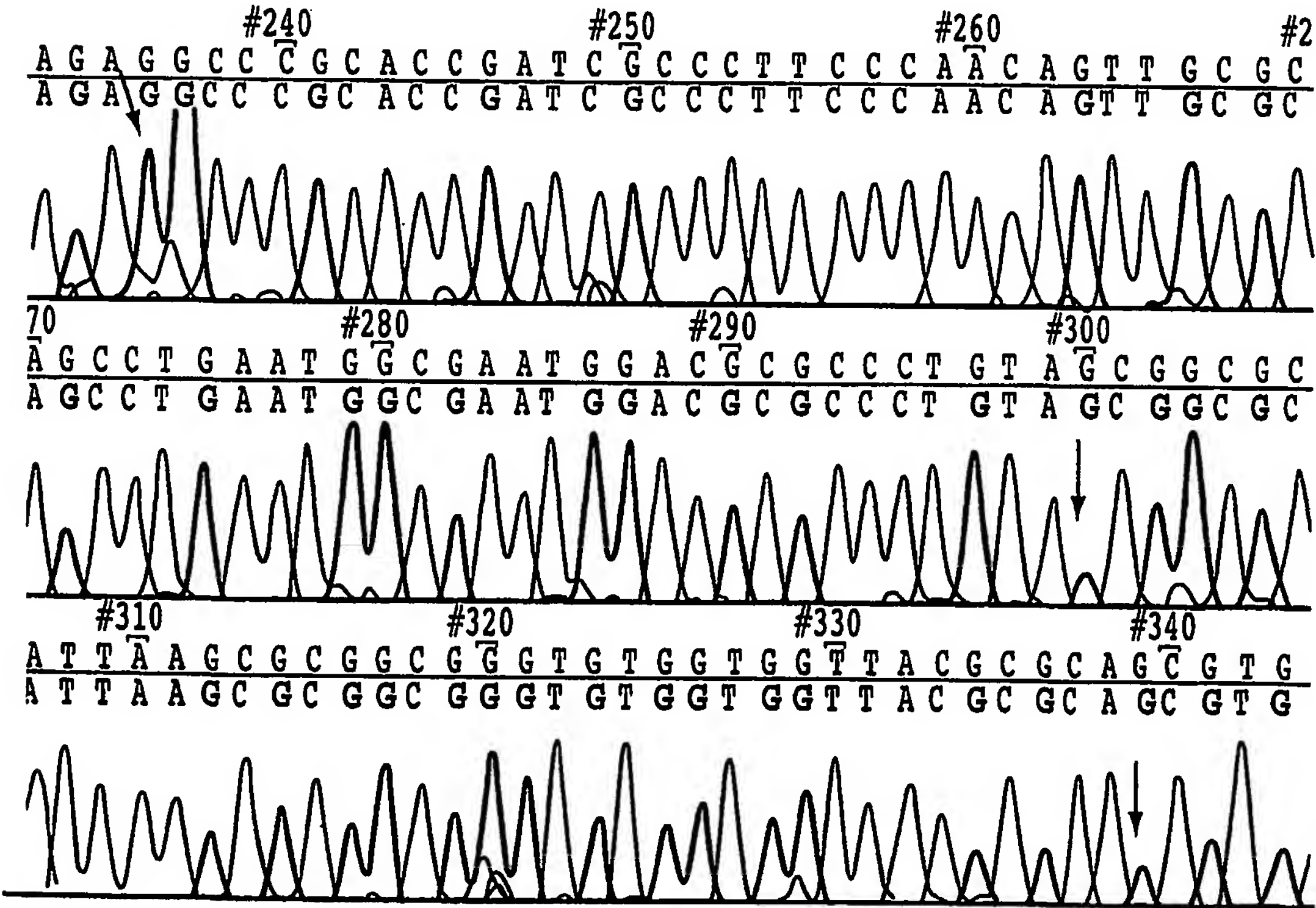


FIG. 4B

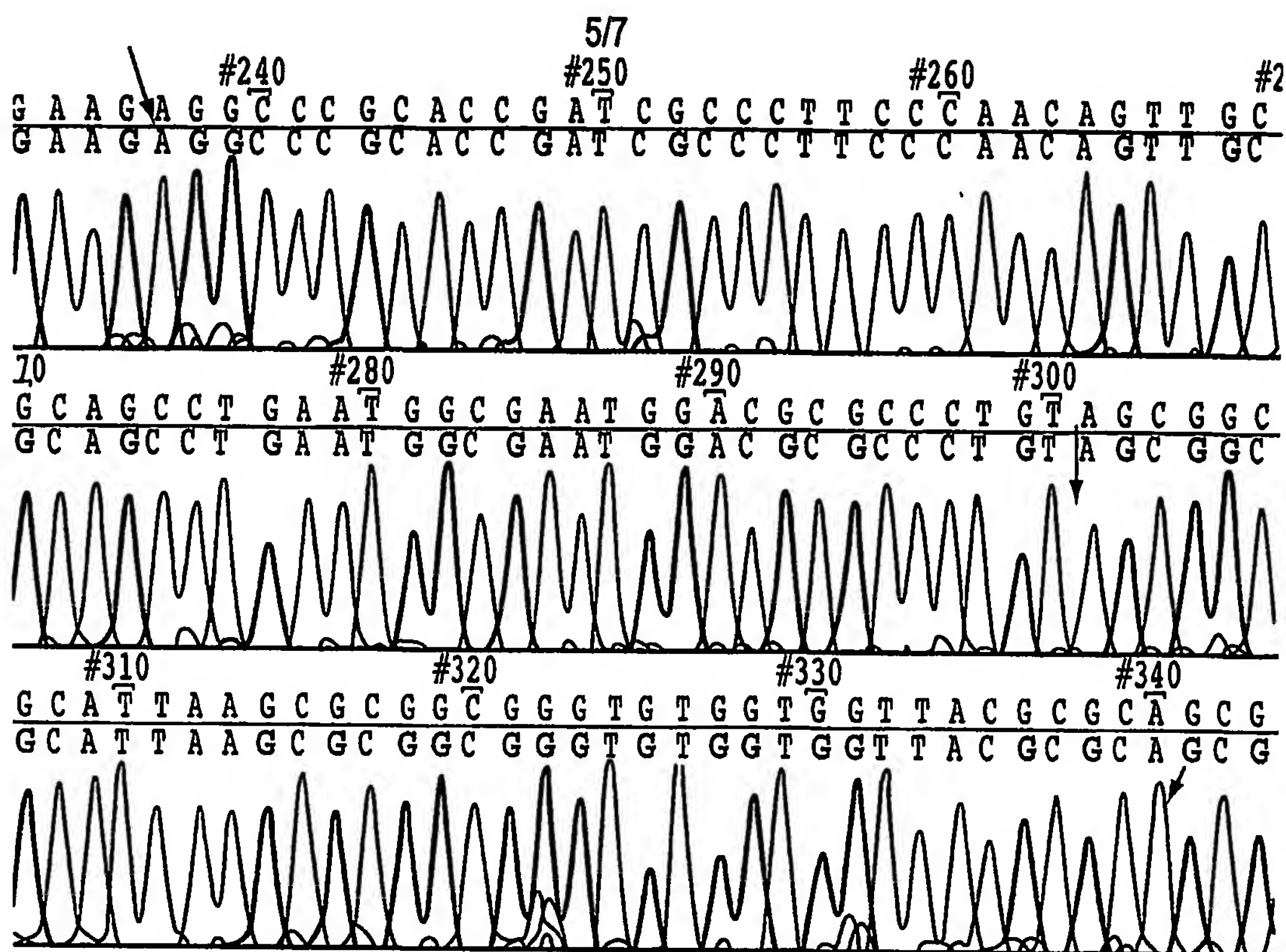


FIG. 4C

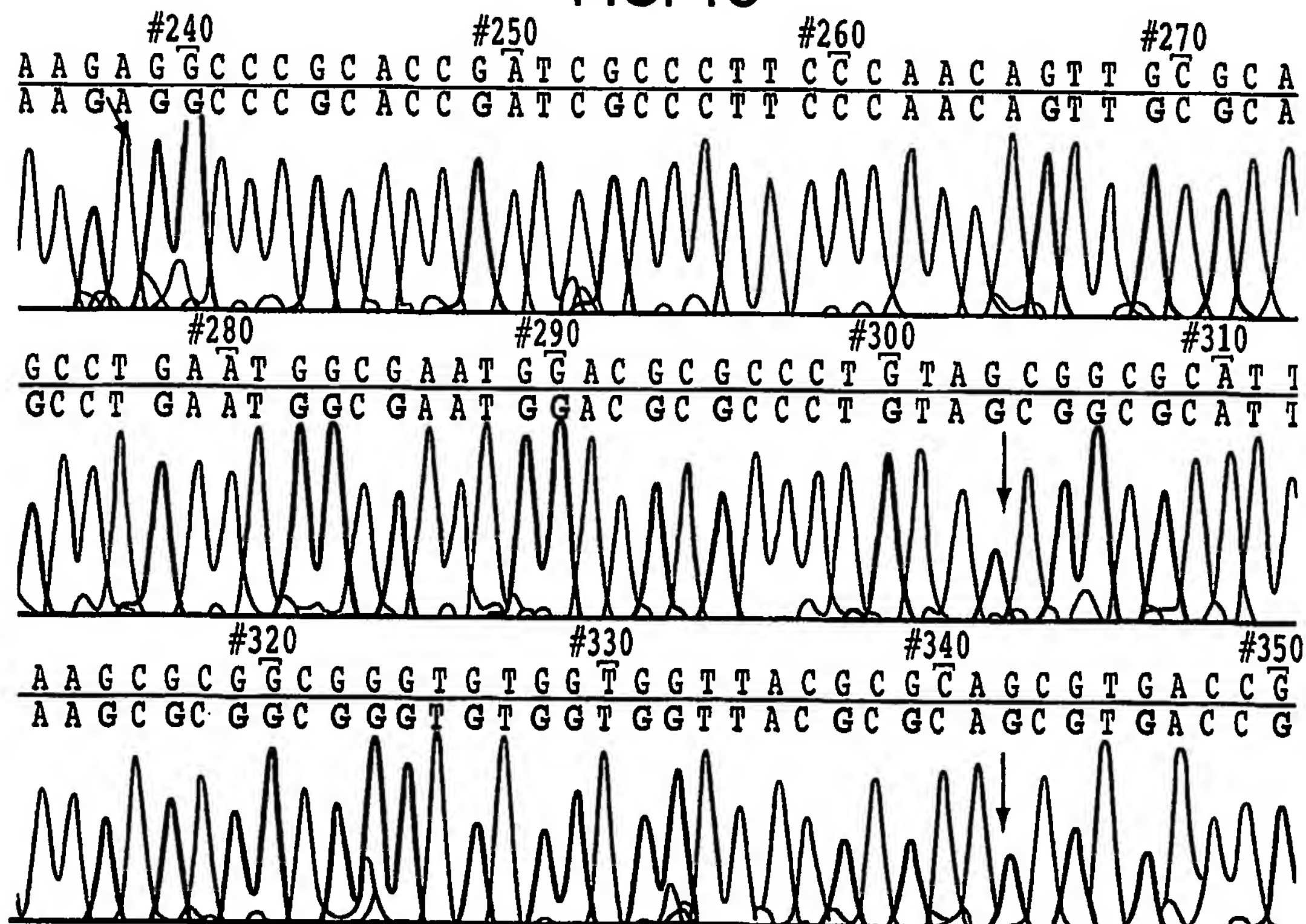


FIG. 4D

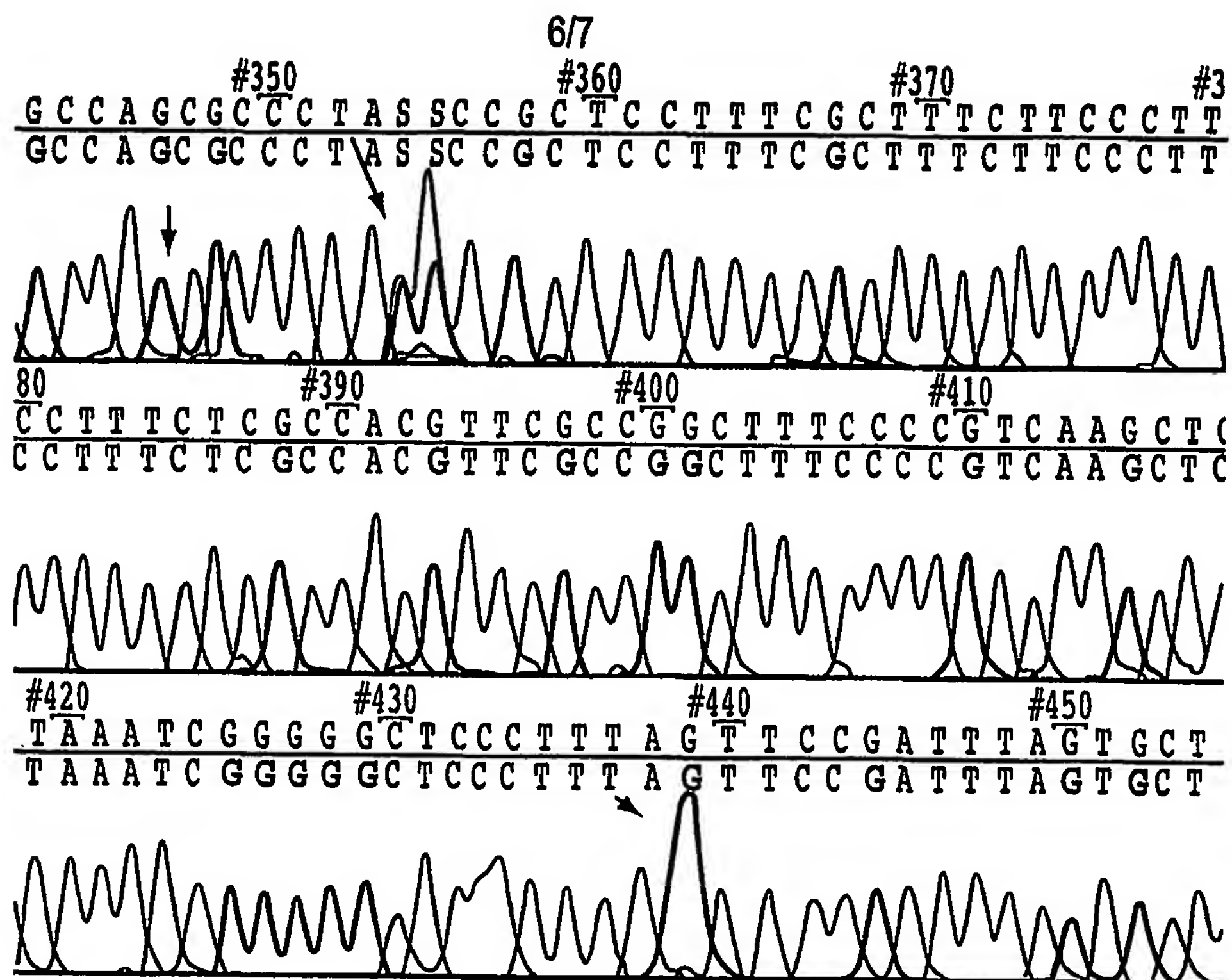


FIG. 5A

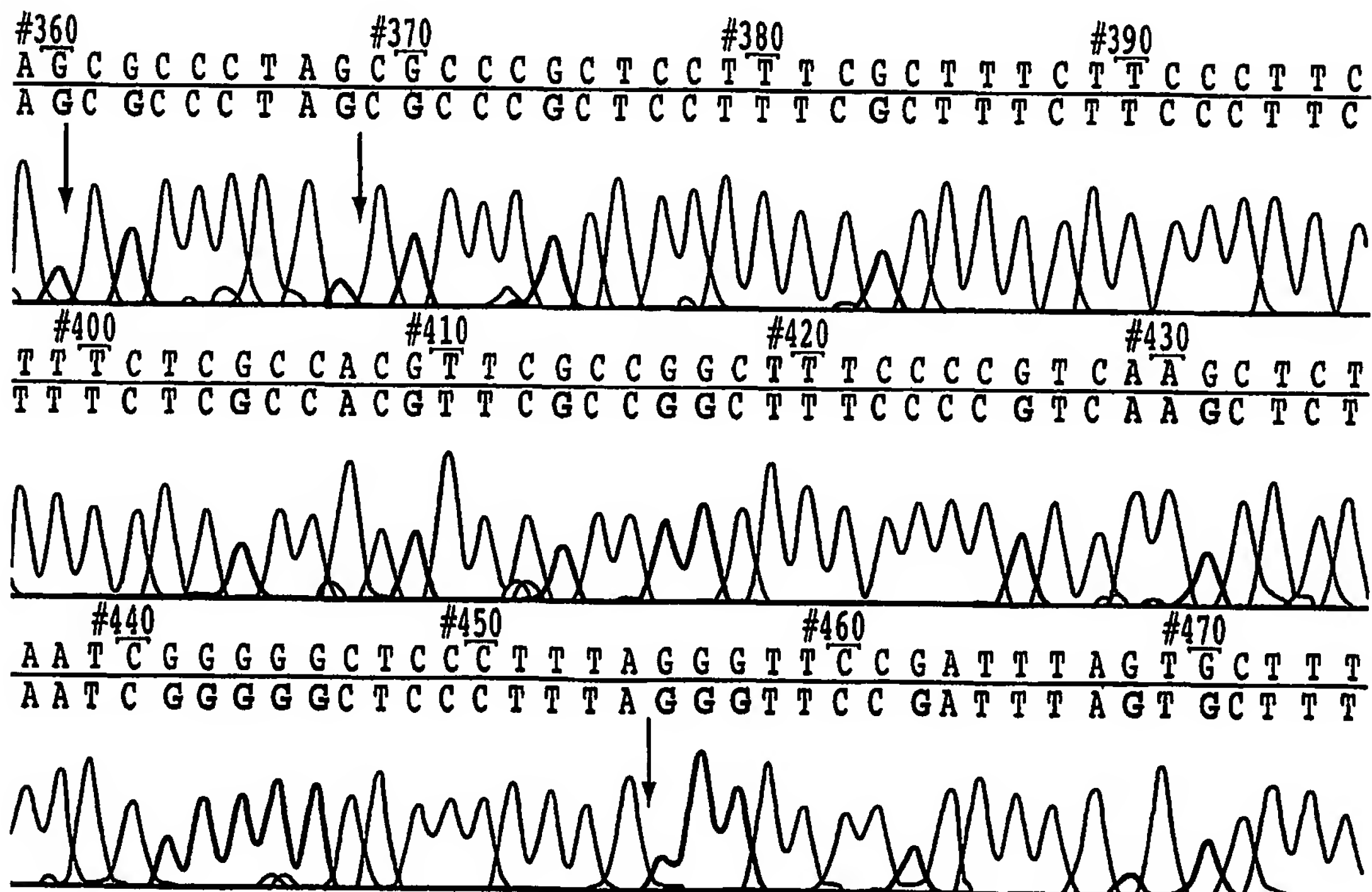


FIG. 5B

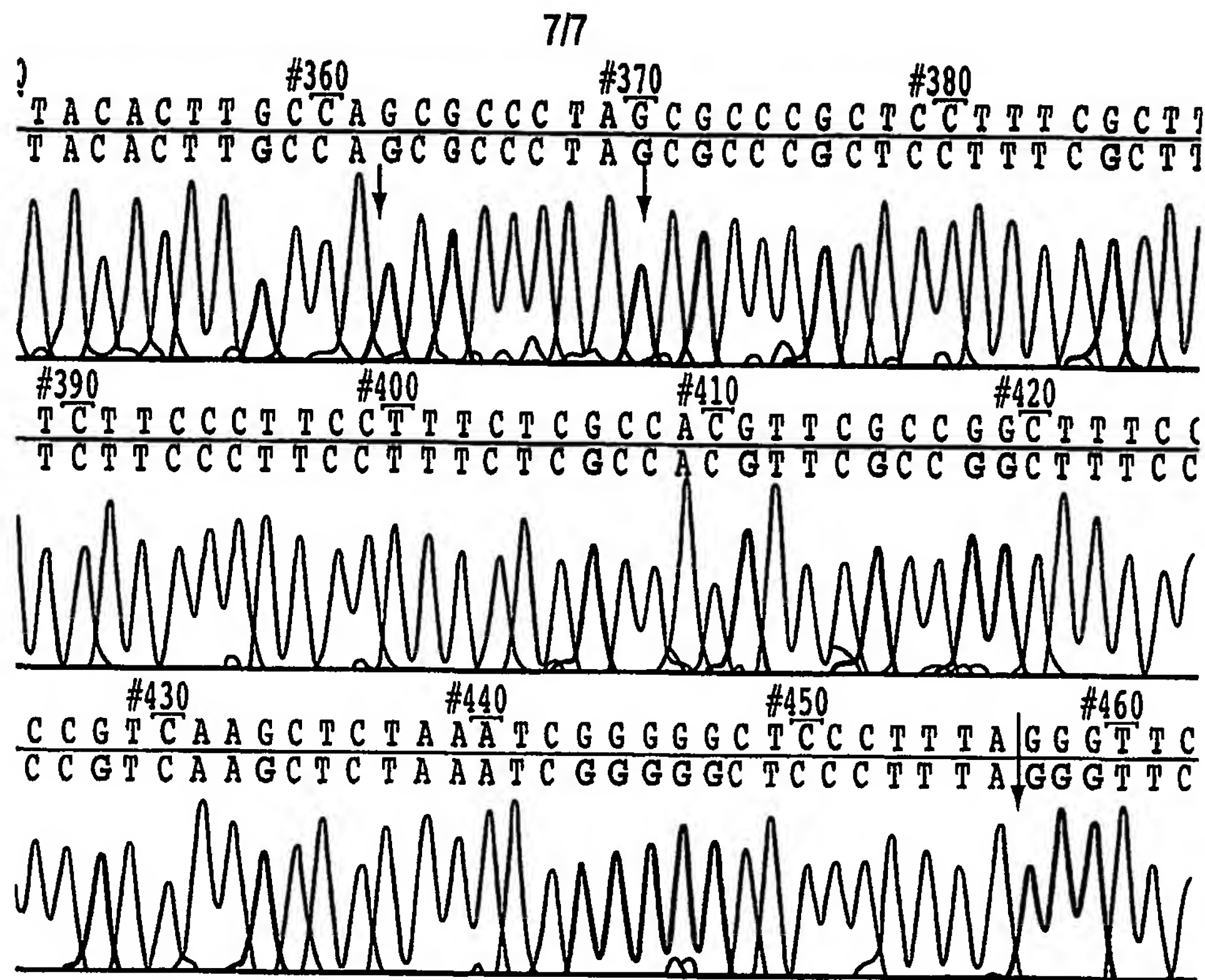


FIG. 5C

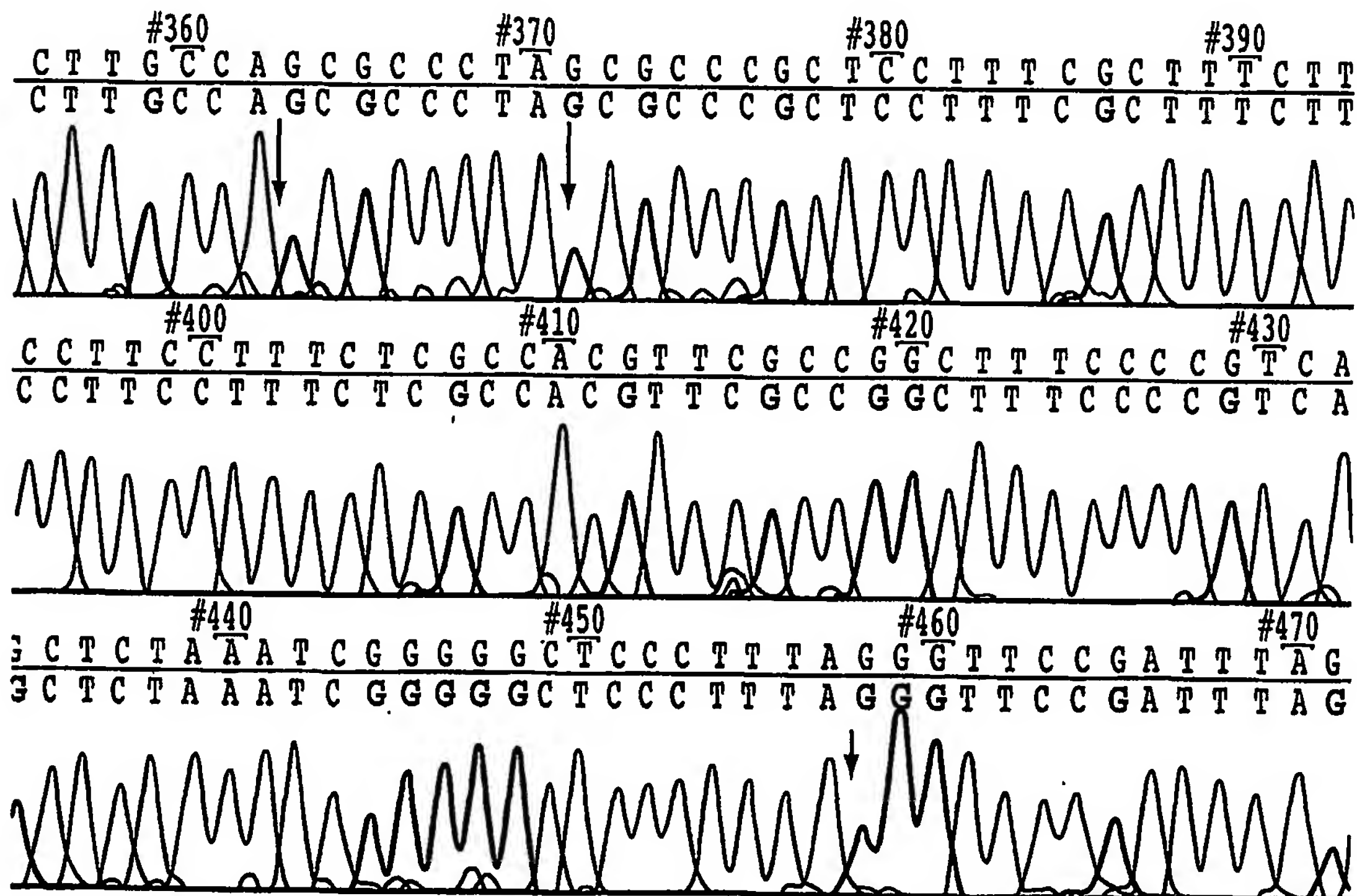


FIG. 5D

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/30455

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12Q 1/68

US CL :435/6; 436/501; 935/19,76,77,78

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6; 436/501; 935/19,76,77,78

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,962,020 A (TABOR et al) 09 October 1990, see entire document especially col. 8, line 56 to col. 9, line 14.	1,4,7
Y		2,3,5,6,8
A	STANLEY TABOR et al. DNA sequence analysis with a modified bacteriophage T7 DNA polymerase. Proceedings of the National Academy of Sciences, USA. July 1987. Vol.84. pages 4767-4771.	1-8
A	N.S. AMBARTSUMY et al. Elimination of the Secondary Structure Effect in Gel Sequencing of Nucleic Acids. June 1980. Vol.114. No. 2. pages 265-268.	1-8



Further documents are listed in the continuation of Box C.



See patent family annex.

"A"	document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"Z"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

29 NOVEMBER 2001

Date of mailing of the international search report

14 DEC 2001

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

JOHN S. STARSIAK JR.

Telephone No. (703) 308-0861

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/30455

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SAEKO MIZUSAWA et al. Improvement of the dideoxy chain termination method of DNA sequencing by use of deoxy-7-deazaguanosine triphosphate in place of dGTP. Nucleic Acids Research. 1986. Vol. 14. No. 3. pages 1319-1324.	1-8
A	DONALD R. MILLS et al. Structure-independent nucleotide sequence analysis. Proceeding of the National Academy of Sciences, USA. Vol. 76. No. 5. pages 2232-2235.	1-8
A	SAMANTHA LI et al. Elimination of band compression in sequencing gels by the use of N4-methyl-2'-deoxycytidine 5'-triphosphate. Nucleic Acids Research. 1993. Vol. 3. No. 11. pages 2709-2714.	1-8
A	ANGELA LEE et al. Improvements in the chain-termination method of DNA sequencing through the use of 7-deaza- 2' -deoxyadenosine. DNA Sequence-J.DNA Sequencing and Mapping. 1991. Vol. 1. pages 233-239.	1-8
A	KIRK W. McCREA et al. Gel Compressions and Artifact Banding Can Be Resolved in the Same DNA Sequence Reaction. Biotechniques. 19903. vol. 15. no. 5. pages 843-844.	1-8

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/80455

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

USPAT, EPOABS, JPOABS, CAPLUS, DERWENT

search terms: sequenc?, DNA, RNA, nucleic, nucleotide(s), polynucleotide(s), oligonucleotide(s), ddITP, dideoxyinosine triphosphate